UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

APR 2 8 2014

MEMORANDUM

SUBJECT: Review of Product Chemistry, Manufacturing Process and Product Performance Information from the Arizona Cotton Research and Protection Council for *Aspergillus flavus* AF36.

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~~**THIS REVIEW CONTAINS FIFRA CONFIDENTIAL BUSINESS INFORMATION**~~

ACTION REQUESTED: To review the product chemistry, new manufacturing process and compare performance of products from that new process to the performance of the currently registered product to reduce seed colonization by aflatoxin producing strains of *Aspergillus flavus*.

CONCLUSIONS: The use of the alternate suggested grain source for growing *Aspergillus flavus* AF36 utilizing the originally described manufacturing process is acceptable. The new manufacturing process can be used to make a new coated seed product when the registration status of the active ingredient is clarified, the raw data, field plot design and analysis for the efficacy trials is submitted and the clearance of the added inert formulation aids is verified. **NOTE:** There is no efficacy data submitted here to expand the labelled use sites to include all corn growing regions or use on almonds and figs for the product of either manufacturing method.

DATA REVIEW RECORD

Active Ingredient: *Aspergillus flavus* strain AF36
Product Name: *Aspergillus flavus* strain AF36
Chemical Number: 006456
Company Name: Arizona Cotton Research and Protection Council
EPA Registration No: 71693-1

Submission Number: S943932
Decision Number: 485146
DP Number: 418507
MRID No: 492563-01 & -02

BACKGROUND: In an April 30, 2013 meeting with BPPD, the Arizona Cotton Research and Protection Council and IR4 discussed a new process of manufacturing their product *Aspergillus flavus* AF36 (AF36 hereafter) and potential expansion of crops treated to include almonds, corn in all corn growing regions, and potentially figs. This submission is intended to address the request for a new manufacturing process as indicated at that meeting.

DISCUSSION: The data provided in this submission are intended to describe two new production systems: one substituting sorghum for wheat in a process identical to the original AF36 manufacturing method and another method using AF36 spore coating of grain. There were also efficacy trials for the products of these two systems. The efficacy results for the old production process with the substitution of sorghum are not as robust as previous efficacy studies. The trials for the sorghum substitute feed stock were based on field residence time of the colonized seed, total sporulation and displacement of toxigenic strains in soil; not reduction of aflatoxin levels in seed of the treated crop. The efficacy trials comparing coated seed product versus the product of the original manufacturing process were only on cotton at 2 Arizona locations. These field studies are not clearly described and the results are presented as bar graphs, not raw data. The results presented grain persistence, total sporulation, soil displacement and aflatoxin levels in cotton seed. There are no efficacy studies for expanding the corn use to more regions or to other crops as discussed in the April 30 meeting.

RECOMMENDATION: The substitution of sorghum for wheat as a feedstock provides a product similar to the currently registered seed colonized AF36 product. The new manufacturing process with seed coating has been shown to reduce aflatoxin levels in cotton at two Arizona locations. Once the status of the source of the active ingredient and the clearance of the new inerts is resolved, the coated seed product should have a label similar to the currently registered product.
NOTE: There are no efficacy data submitted here to expand the labelled use sites to include all corn growing regions or use on almonds and figs for the product of either manufacturing method.

SUMMARY OF DATA SUBMITTED:

MRID 492563-01: A new seed coating technique was described which employed many of the same starting materials and scale-up steps as the original production method. Collection of the spores from the colonized seed product for coating and formulation with some inerts onto another grain matrix is a step missing in the original manufacturing description.

CLASSIFICATION: Supplemental.
The collection and use of spores from the inoculated grain makes this product different from the originally registered product. Since there is no registration of the technical grade of the AF36 active ingredient, the new manufacturing process for coated seed appears to be using an unregistered

source of the active ingredient. The clearance of the proposed inert ingredients employed in the new manufacturing process cannot be verified.

MRID 492563-02: There is a description of a manufacturing process employing sorghum as the growth matrix for a product using the same multiple step process with dry down and packaging of colonized sorghum seed as the final product. This substitution of grain sorghum for wheat has been shown to have similar persistence and sporulation potential as the original wheat based product. The data for the efficacy of the new seed coated product is less robust. There is an indication that the coated sorghum product produces more spores per gram of product compared to the original manufacturing process with colonized wheat. The efficacy data on cotton from two Arizona locations suggests that the coated sorghum product can perform similarly to the original colonized wheat seed product at replacing the aflatoxin producing S strains and reducing aflatoxin levels in cotton seed.

CLASSIFICATION: Supplemental.
Sorghum as substrate for the colonized seed production method provides a similar AF36 product. The new AF36 coated seed manufacturing process requires resolution of the AF36 spores registration as an active ingredient and clearance of the inert formulation aids. **NOTE:** There are no efficacy data submitted here to expand the labelled use sites to include all corn growing regions or use on almonds and figs for the product of either manufacturing method.

DATA EVALUATION REPORT

Reviewed by: John L. Kough, Ph.D., Biologist, BPPD

STUDY TYPE:	Product Chemistry and Product Performance for *Aspergillus flavus* AF36 Prevail End-use Product
MRID NO:	492563-01; 492563-02
TEST MATERIAL:	Sorghum coated with spores of *Aspergillus flavus* AF36 for competitive exclusion and control of aflatoxin producing *A. flavus*
STUDY NO:	IR-4 PR# 0052B
SPONSOR:	Arizona Cotton Research and Protection Council, Phoenix, AZ
TEST FACILITY:	Arizona Cotton Research and Protection Council, Phoenix, AZ
TITLE OF REPORT:	Product Chemistry for *Aspergillus flavus* AF36 End-use Product (OSCPP Guidelines 885.1100- 880.1400) & Product Performance for *Aspergillus flavus* AF36 Prevail End-use Product.
EPA REG. NO.:	71693-1
AUTHOR:	Peter Cotty
STUDY COMPLETED:	November 1, 2013

CONCLUSION: A new seed coating technique was described which employed many of the same starting materials and scale-up steps as the original production method. Collection of the spores from the colonized seed product for coating and formulation with some inerts onto another grain matrix is a step missing in the original manufacturing description. There is a description of a manufacturing process employing sorghum as the growth matrix for a product using the same multiple step process with dry down and packaging of colonized sorghum seed as the final product. This substitution of grain sorghum for wheat has been shown to have similar persistence and sporulation potential as the original wheat based product. The data for the efficacy of the new seed coated product is less robust. There is an indication that the coated sorghum product produces more spores per gram of product compared to the original manufacturing process with colonized wheat. The efficacy data on cotton from two Arizona locations suggests that the coated sorghum product can perform similarly to the original colonized wheat seed product at replacing the aflatoxin producing S strains and reducing aflatoxin levels in cotton seed.

CLASSIFICATION:	SUPPLEMENTAL. The use of *Aspergillus flavus* AF36 spores as the source of the active ingredient in the alternate manufacturing process needs to be clarified. The two inerts do not appear to be cleared for the intended use. The study design and raw data for the efficacy studies should be provided for the new manufacturing process using seed coating technology.
GLP STATEMENT:	A statement that these studies do not meet 40CFR160 GLP standards and that data presented in this study were not conducted under GLP.

Study Design

Three products are tested in the submitted studies; each using different manufacturing procedures. One is the currently registered product based on AF36 colonization of wheat seed, the second

product uses the same seed colonization technology substituting sorghum for wheat and the last is termed the "new process" which uses colonized wheat seed to produce spore preparations that are subsequently coated onto pasteurized sorghum. The intent of the submission was to compare the three AF36 products as being similar and of equivalent efficacy against aflatoxin producing *A. flavus* strains.

Test Material: *Aspergillus flavus* AF36.

I. PRODUCT IDENTITY: The new formulation contains the active ingredient at 0.0008% of the new end-use product named *Aspergillus flavus* AF36 Prevail.

Deficiencies: The status of the currently registered AF36 product (manufactured as an integrated process) as the source of AF36 spores needs to be clarified.

References:
MRID 437634-01.
MRID 439900-01.

II. MANUFACTURING PROCESS: see confidential appendix.

Deficiencies: None.

III. DISCUSSION OF FORMATION OF UNINTENTIONAL INGREDIENTS: No data or rationale was submitted to address the end-use product, just the methods used for the currently registered product.

Deficiencies: This section was not addressed for the revised manufacturing process. The acceptance and rejection criteria for contamination needs to be included and at what step these are performed.

IV. ANALYSIS OF SAMPLES: A process that measures turbidity of a spore suspension in sterile water and compares the nephelometric turbidity units to a colony forming unit curve is used. The suspension dilution is adjusted to achieve 1 to $5x10^7$ spores in 7 mL to be added to 1.0 Kg of grain.

Deficiencies: None.

References:
MRID 468595-01.

V. CERTIFICATION OF LIMITS: see confidential appendix.

VI. PHYSICAL AND CHEMICAL CHARACTERISTICS: Methods and results are in Table 1.

TABLE 1. Physical and Chemical Properties for PREVAIL.		
Guideline Reference No./Property	Description of Result	Methods
830.6302 Color	Blue.	Observation.
830.6303 Physical State	Solid.	
830.6304 Odor	Grain odor.	
830.6313 Stability	N/A.	
830.6317 Storage Stability	Preliminary data covers 6 months.	
830.6319 Miscibility	N/A – not an emulsifiable liquid.	
830.6320 Corrosion Characteristics	Grain is non-corrosive.	
830.7000 pH	N/A.	
830.7100 Viscosity	N/A.	
830.7300 Density/Relative Density/Bulk Density	700-800 Kg/M^3	Gravimetric measurement.

[a]Data from MRID 492563-01.

Storage Stability Testing: Approximately 6 months elapsed from the production assay and retesting of a single batch; a bar graph (N=4) demonstrates that the samples contained approximately $3.0x10^9$ spores/g.

Deficiencies: Only one batch was assayed; potential for contaminants was not addressed.

VII: PRODUCT PERFORMANCE: The use of sorghum as an alternate growth substrate for the original manufacturing process was addressed by graphical data containing error bars collected from several sites in Arizona in cotton- and maize-growing fields. The field data was generated sometime in 2007 and 2008; no certificate of analysis for the pesticide test batches was provided for comparison to the newly submitted manufacturing process nor on how the wheat- and sorghum-variant characteristics were compared at manufacture. Minimal descriptions of the field sites and test methods were provided. The opening paragraph indicates the tested pesticides were produced using the previous manufacturing process, though with sorghum rather than wheat. Aflatoxin analyses are not reported.

Deficiencies: Details of experimental design and locations were not clear. Data do not support the new seed coating manufacturing process; simply substitution of sorghum for wheat.

VII. PRODUCT PERFORMANCE: The use of the product of the new manufacturing process of seed coating was tested on cotton at two locations in Arizona (Wellton and Casa Grande). The measured endpoints included recovered grains, sporulating grains, and spore produced after 10 days. There was also an accounting of replacement of S strains (aflatoxin producers) with AF36 tracking percentages at pre-treatment, following treatment and on harvested cotton seed. The actual level of AF36 and S strain in soil and harvested cotton seeds was also tracked as were the aflatoxin levels on the harvested cotton seed. While none of the untreated control plots had levels of aflatoxin that exceeded the FDA action level, there were reductions in the aflatoxin levels associated with AF36 treatment. Finally, there was a laboratory study showing similar rates of spore production for two batches of sorghum coated AF36 product (84P74 and 85Y34) compared to a colonized wheat formulation.

FIFRA CBI APPENDIX

Manufacturing process information may be entitled to confidential treatment

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